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2.	Patent application number *(The Patent Office will fill in this part)*	9917299.1
		23 JUL 1999
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	TEPNEL MEDICAL LIMITED UNIT 8, ST GEORGE'S COURT HANOVER BUSINESS PARK ALTRINCHAM WA14 5UA
	Patents ADP number *(if you know it)*	1444 9SL2002
	If the applicant is a corporate body, give the country/state of its incorporation	
4.	Title of the invention	ISOLATION OF BIOMOLECULES
5.	Name of your agent *(if you have one)*	Marks & Clerk
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Sussex House 83-85 Mosley Street Manchester M2 3LG
	Patents ADP number *(if you know it)*	18004 ✓

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7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		Number of earlier application	Date of filing *(day/month/year)*
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ISOLATION OF BIOMOLECULES

The present invention relates to a purification method and apparatus for use in obtaining samples of biomolecules. The invention relates more particularly, but not exclusively, to such method and apparatus which may be used for obtaining samples of nucleic acids or proteins.

Numerous methods are known for obtaining biomolecules, for example nucleic acids and proteins, from biological material such as viruses, bacterial and eukaryotic cells, cell aggregates and tissue or body fluids. Typically the biomolecule to be obtained is a soluble molecule and is "released" from the biological material by a lysis procedure (e.g. alkaline lysis) resulting in a suspension comprised of a solution of the target biomolecule also containing soluble proteins, carbohydrates, fats, amino acids and other metabolites from the disrupted cells.

In many methods known in the art for effecting the purification of a desired biomolecule, those molecules which would otherwise contaminate the desired product are insoluble, or are rendered so by a chemical process. The insoluble material is then removed by methods known in the art (e.g. centrifugation and aspiration of the supernatant) to achieve a degree of purification of the soluble material. These insoluble materials may include, for example, whole cells, or fragments thereof, flocculated proteins and unwanted nucleic acid material (e.g. chromosomal DNA contamination of a plasmid DNA preparation).

After separation of the insoluble material, from the solution, the latter is applied to a solid phase binding matrix under conditions (e.g. in the presence of a chaotropic salt) such that the matrix binds the biomolecule of interest. Subsequently the solution is removed from the matrix (leaving the biomolecule bound thereto), the matrix washed to remove non-bound material, and the biomolecule eluted.

The separation of the insoluble material from the solution is generally effected by centrifugation of the suspension followed by careful pipetting. However the use of centrifugation is a disadvantage in that it does not easily allow the procedure for obtaining the biomolecule to be fully automated. Furthermore, the separation of soluble from insoluble material by pipette is awkward to perform and must be carried out accurately to prevent unwanted insoluble material from being added to the solid phase matrix and causing contamination.

One purification method which seeks to overcome some of the problems associated with conventional techniques is disclosed in WO-A-95/02049. The apparatus disclosed involves a pneumatic delivery system which is used to add, mix and remove reagents from a flow-through vessel in order to separate a target biomolecule from cells. The vessel has two chambers between which is a porous membrane or filter. The membrane functions to retain cells and cellular debris and insoluble material in the upper chamber whilst soluble material is filtered through and further purified by binding to a solid phase matrix present in the lower chamber.

There are however disadvantages associated with the technique disclosed in WO-A-95/02049. In particular, if it is desired to treat the solid phase matrix (with biomolecule bound thereto) with additional solutions (e.g. wash or elution solutions) it is necessary to pass those solutions into the lower chamber either via the upper chamber and filter (which retains insoluble material) or provide additional inlets in the lower chamber. In the former case, there is the risk of contamination. In the latter case, the provision of additional ports in the lower chambers makes the apparatus difficult to manufacture.

It is an object of the present invention to obviate or mitigate the aforementioned disadvantages.

According to a first aspect of the present invention there is provided a method of obtaining a sample containing the biomolecule from a suspension comprising a solution of the biomolecule and insoluble material, the method comprising the steps of:

(a) providing a biomolecule purification assembly comprised of a vessel having a liquid inlet and of a filter unit removably located on the liquid inlet;

(b) effecting a filtration of the suspension through the filter unit so as to cause the solution to enter the vessel through the liquid inlet;

(c) removing the filter unit from the liquid inlet;

(d) immobilising the biomolecule on a solid phase support; and

(e) subjecting the molecule to at least one of the steps of washing on the support and elution from the support to obtain a purified sample of the biomolecule.

According to a second aspect of the present invention there is provided apparatus for obtaining a sample of a biomolecule from a suspension comprising a solution of the biomolecule and insoluble material, the apparatus comprising

(i) a biomolecule purification assembly comprised of a vessel having a liquid inlet and of a filter unit removably located on the liquid inlet of the vessel;

(ii) means for causing the solution present in the suspension to pass through the filter unit and the liquid inlet into the vessel; and

(iii) means for removing the filter unit from the vessel.

In accordance with the invention therefore separation of insoluble material from the suspension is effected through a filter unit which is removably located in the liquid inlet of a vessel and a filtration is effected causing the solution to enter the vessel. Any insoluble impurities in the suspension are retained on the filter unit. Subsequently, the filter is removed from the inlet and is preferably discarded rather than being reused. Thus each filter need only be used once avoiding problems of contamination.

The method of the invention negates the requirement for the suspension to be centrifuged to separate soluble and insoluble materials in the suspension. The method further negates the requirement for accurate separation of the soluble and insoluble phases by use of a pipette. Furthermore, the method of the invention is relatively simple to perform and is eminently suited to automation as will be appreciated from the description given below.

It is preferred that, in step (e) of the method, the step of washing or elution is effected on solid phase support contained within the vessel. Preferably both of the steps of washing and elution are effected, and the biomolecule of interest is eluted through said inlet of the vessel.

It is particularly preferred that the solid phase support material is a particulate or bead-like material which is introduced into the vessel (preferably through said inlet) after the step of removing the filter unit therefrom.

The vessel of the biomolecule purification assembly is preferably a flow through vessel, most preferably an open-ended column, e.g. having a volume sufficient to hold 0.25 to 1.5ml of sample, disposed vertically so that its lower end provides the aforementioned liquid inlet and the upper end may be used for the introduction of additional reagents into the column as required.

The vessel may comprise upper and lower bore sections whereof the diameter of the upper section is greater than that of the lower section, the two sections being connected by a intermediate, tapering bore section, the purpose of the reduced section lower bore will be described below.

The filter unit may be of any material capable of tolerating the reagents used and will comprise a filter element having a pore size which is capable of preventing passage of the insoluble material of the composition therethrough but which is not so small that the flow rate through the filter becomes unacceptably low. Typically the pore size of the element will be in the range 0.2 to 50 microns.

The filter unit may, for example, comprise a sleeve or the like for location over the liquid inlet of the vessel and a filter located in the sleeve. The filter unit may for example be a push fit or a loose snap fit over the liquid inlet.

Preferably the filter incorporates a depression locating in close proximity to the liquid inlet 8 of the vessel. This allows filtrate to come into close proximity with the inlet and enhance the rate of filtrate uptake into the vessel.

The filtration step of the method of the invention may be effected in a number of ways. Thus for example, a reduced pressure may be applied to the interior of the vessel causing solution from the suspension to be drawn through the filter unit into the vessel. For example, when the biomolecule of interest is a nucleic acid molecule a chaotropic salt will generally be required to allow the nucleic acid molecule to bind to the matrix. The chaotropic salt may be provided with the suspension to be applied to the matrix or may be pre-equilibrated with the matrix prior to contact of the filtered solution with the matrix. Subsequently, excess solution may be discharged from the vessel.

As an alternative to the use of reduced pressure, the suspension may be provided in an open-topped container within which the filter unit is a close sliding fit such that by moving the filter unit within the container towards the base thereof solution is caused to be forced through the filter unit into the vessel for binding of the biomolecule as described above.

Subsequent to the filtration operation, the filter unit is removed from the vessel.

It is particularly preferred in accordance with the invention that the suspension to be filtered is contained in a well and that once the filtration operation is complete the filter unit is automatically discarded into that well. This may be achieved in a number of ways. Thus, for example, the biomolecule purification assembly may be lowered towards the well to effect filtration and move upwardly after filtration is completed and the apparatus may incorporate a stripping arrangement which acts on the filter unit as the biomolecule purification assembly is moved upwardly causing the filter unit to be discharged into the well. Alternatively, the filter unit and microtitre well have inter-engagable formations whereby as the biomolecule purification assembly is lowered towards said well the formations come into an engaging relationship requiring a greater force to release the engagement than is required for removing the filter unit from the vessel. Thus, as the vessel is moved upwardly away from the well, the engagement is maintained and the filter unit is removed from the vessel and retained in the well.

Subsequent to the removal of the filter unit, the biomolecule is immobilised in a solid phase support (i.e. step (d) of the method), preferably in the form of particles or beads having a size of 0.1 to 250 microns Preferably the particles do not fill the entire space between the retaining means so that the particles may be "fluidised" within the vessel.

The immobilisation of the biomolecule onto the solid phase support is preferably effected in the presence of a chaotropic salt or other agent(s) capable of effecting absorption of the biomolcule onto the support. By chaotropic salt it is meant any substance capable of altering the secondary, tertiary and/or quaternary structure of a protein or nucleic acid molecule, but leaving at least the primary structure intact. Examples of chaotropic salts which may be utilised to allow binding of nucleic acid of proteins to the solid phase binding matrix are guanadinium salt, sodium iodide, potassium iodide, sodium (iso)thiocyanate, urea or combinations thereof. Preferred chaotropic salts for use in the present invention include guanidinium hydrochloride and guanidinium (iso)thiocyanate. For the purposes of effecting step (d) of the method (i.e. immobilising the biomolecule on the solid phase support), the filtrate in the vessel may contain the chaotropic salt or other immobilising agent(s) and the filtrate is discharged onto the support material prior to the resultant mixture being taken back into the vessel. Alternatively, the filtrate may be discharged into a mixture of the support and the chaotropic salt or other agent(s) and the resultant mixture is then taken back into the vessel.

Subsequently, the mixture of the filtrate. chaotropic salt (or other immobilising agent(s)) and the beads may be drawn back up into the vessel and, if desired, may be subject to at least one cycle of discharge from, and uptake back into, the vessel to improve mixing.

It is preferred that the support comprises magnetic beads.

The magnetic beads within the vessel may be manipulated by a magnet positioned externally of the vessel.

Step (e) of the method may be effected in a number of ways.

Thus, for example, a magnet may be used to "hold" the magnetic beads (with immobilised biomolecule) at the side of the vessel. In the case of the preferred biomolecule purification assembly in which the vessel has a reduced diameter bore section, it is preferred that the beads are "held" at this position in the vessel.

Subsequently, the solution may be discharged from the vessel, wash buffer introduced into the vessel, and the magnet manipulated to re-suspend the beads in the buffer. The magnet may once again be used to hold the beads in place and the wash buffer discharged from the vessel.

The washing operation may be repeated at least once. For preference the final wash step employs 70% ethanol (in water).

After removal of the final wash solution from the vessel, (and with the beads being held therein by means of the magnet), air may be passed over the beads to effect drying. Alternatively or additionally heat may be applied to the vessel, e.g. by locating the latter in a heating block.

Subsequently, the biomolecule may be eluted from the vessel. This may be achieved by introducing an elution buffer into the vessel, admixing the particles with the buffer, heating the admixture, immobilising the particles by means of the magnet, and discharging the solution (containing dissolved biomolecule) from the vessel for collection and subsequent processing

A particularly preferred embodiment of apparatus in accordance with the invention is capable of handling an array of biomolecule purification assemblies and therefore each of the individual steps (described above) of filtration, removal of the filter unit, "pick up" of magnetic particles, washing and elution is effected simultaneously on all members of the array.

In such an apparatus, it is preferred that the vessels (of the biomolecule purification assemblies) are vertically disposed, open-ended columns. The upper ends of such columns may be associated with pumps for applying reduced pressure to the interior of the column for drawing liquid into the columns (e.g. for the purposes of the filtration and washing and elution operations) and for blowing or drawing drying air through the columns. Furthermore, the upper ends of the vessels may be associated, via appropriate valving arrangements, with appropriate reagent reservoirs permitting reagents to be passed downwardly into the columns if required.

A particularly preferred embodiment of apparatus in accordance with the invention for use in conjunction biomolecule purification assembly's as defined in the previous paragraph comprises separate filtration, bead "pick up", washing and elution stations Thus, at the filtration station, there may be a first set of wells (e.g. a BioBlock) each containing an aliquot of the suspension and each member of the array effects filtration of an aliquot from the corresponding well at the station. The apparatus may be such that the filter unit of the biomolecule purification assembly is discharged into the respective well at the filtration station. At the bead "pick up" station, there may be a second set of microtitre wells each containing magnetic beads in a chaotropic solution. At the wash station, wash solution may be passed to the vessel (e.g. by pumping) and discharged through the liquid inlet into a waste receptacle which may be connected to a drain. Furthermore, the elution station may have a second set of microtitre wells containing elution buffer into which the filtrate/magnetic beads are discharged prior to being taken back up into the vessel. The final step of elution is then effected by holding the magnetic beads in position and discharging the liquid containing the desorbed biomolecule from the vessel. Obviously, the apparatus will include a magnet or magnets as necessary for manipulating the magnetic beads.

In a particularly preferred embodiment of the apparatus as described in the previous paragraph, the apparatus incorporates a head arrangement capable of selectively "picking-up" and releasing 12 of the biomolecule purification assemblies.

Furthermore. the wells at the various stations may each be provided by a 12 by 8 array of microtitre wells so that in any one cycle of the apparatus a total of 12 samples may be processed. By operating the apparatus through 8 cycles then a total of 96 samples may be processed before the microtitre wells need to be replaced.

It is also preferred that the apparatus incorporates an upstream station at which the head arrangement is capable of "picking-up" the biomolecule purification assemblies to be used in any one cycle of the apparatus and a final discharge station at which the vessels of the assemblies are discarded. Thus, if the head arrangement is capable of holding 12 biomolecule purification assemblies then 12 such assemblies are "picked-up" at the beginning of each cycle of the machine.

Such an apparatus will further comprise mechanisms for moving those parts of the apparatus as are required to complete the method of the invention in the directions (X,Y and/or Z) so to do. The apparatus as described above may readily be automated and is capable of operating under the control of a programmed microprocessor.

The method and apparatus of the invention are particularly suitable for obtaining a sample of a biomolecule from a suspension obtained by a lysis procedure (e.g. a standard alkaline lysis procedure as well known to those skilled in the art) effected on a biological material.

The target biomolecule may, for example, be a nucleic acid (DNA or RNA) and may for example be a semi-purified or non purified, native or synthesised nucleic acid. The target soluble biomolecule may be any DNA or RNA sequence from a viral, bacterial, animal or plant source. Apart from the utility in purifying DNA and RNA samples and especially for purifying plasmid DNA and other recombinant DNA constructs, such as phagemids, free from chromosomal DNA, the method and

apparatus according to the present invention are also suitable for isolating recombinant proteins and antibodies, especially from cellular samples

The biological material on which the lysis is effected may for example comprise cells. For the purposes of the present specification, the term "cell" is intended to encompass bacterial cells, cells (e.g. blood cells) from higher organisms, virus particles and other cell types or organelles which contain the target biomolecule and which may be released in a soluble form by a lysis procedure.

In the case of bacteria, the nucleic acid to be isolated may be from the bacterial genome or from an extragenomic element such as a plasmid. Phage infected bacteria may also be used for the preparation of phage DNA, such as M13 DNA.

According to a preferred embodiment of the present invention the composition from which the target biomolecule is to be isolated is a lysed and neutralised bacterial cell composition containing soluble plasmid DNA and insoluble precipitated genomic DNA, flocculated protein and other cellular debris.

In order to isolate plasmid DNA that has been propagated within bacterial hosts, the following steps may be followed:

i) growth of bacterial host in an enriched medium;

ii) centrifugation of bacteria to form a pellet after which the supernatant is discarded;

iii) resuspension of the bacterial pellet in a buffered solution;

iv) addition of lysis reagent which releases the cellular contents; and

v) addition of neutralisation solution which causes the formation of suspension comprising a solution containing dissolved plasmid.

In a particularly advantageous implementation of the invention, the resuspension obtained from step (iii) may be provided, e.g. in a microtitre well, to the

apparatus which is adapted to be such as to add lysis solution to the resuspension. In the preferred embodiment of the invention, in which the vessels (of the biomolecule purification assemblies) are vertically disposed, open-ended columns, the apparatus comprises a 'column-head' on which the biomolecule purification assemblies are to be mounted. However, prior to mounting of the assemblies on their respective heads, lysis solution may be injected ("fired") from the head into the resuspension in the micro-titre well. The neutralising solution may be added in the same way. This will also provide for good mixing of the lysis solution and neutralising solution with the resuspension. Subsequently, the biomolecule purification assemblies are mounted on their respective heads for effecting separation, washing and elution procedures as described.

In an alternative implementation, the lysis solution (drawn from a reservoir thereof) may be ejected downwardly through the column into the resuspension prior to mounting of the filter unit on the column.

In both cases the inventors have found that introduction of the lysis solution and neutralising solution in this way into the resuspension avoids the need for any further mixing so lysis and neutralisation can be effected. Furthermore, the introduction of the lysis and neutralising solution is effected without the dispensing apparatus coming into contact with the sample or its containing vessel.

In a particularly preferred embodiment of the invention firing of multiple aliquots of lysis solution or neutralising solution into the resuspension well provides better mixing (or at least more efficient extraction of product) than a single addition of reagent.

The invention will be further described, by way of example only, with reference to the accompanying drawings, in which:

Fig. 1 schematically illustrates a first embodiment of the invention;

Fig 2 schematically illustrates the column of the assembly shown in Fig 1;

Fig 3 illustrates the sleeve of the filter unit in Fig 1; and

Fig. 4 schematically illustrates to an enlarged scale the filter of the assembly shown in Fig 1.

Referring firstly to Fig. 1, there is illustrated a biomolecule purification assembly 1 for use in obtaining a purified sample of a biomolecule of interest from a suspension (e.g. as obtained by an alkaline lysis procedure) comprising a solution of the biomolecule containing insoluble biological debris. The illustrated assembly 1 comprises a vertically disposed. open-ended column 2 and a filter unit 3.

Referring to Fig 2, column 2 is referenced for convenience as being comprised of body sections 4, 5, 6 and 7. Body section 4 defines an upper cylindrical bore 4a which at its lower end is connected to a downwardly tapering section 5a leading into a lower bore 6a which is of reduced diameter as compared to bore 4a. At its lower end bore 6a leads into a tapering section 7a defined within the lower section 7 of the column 2.

The lower end of tapering section 7a defines a liquid inlet 8 for the column.

At the upper end of column 2 there is provided a formation 9 by means of which the column may be mounted on the head of a sample processing apparatus of the type described more fully above.

Referring back to Fig 1, the filter unit 3, is a two component part and comprises a sleeve 10 (see Fig 3) having an internal bore 11 within which is housed a filter 12(see Fig 4) having depression 13 in its upper surface. Filter 11 is such that it is permeable to liquids but is capable of filtering the insoluble debris in the suspension from which the biomolecule is to be obtained.

Sleeve 10 is removably mounted on column 2 and more particularly is located over section 7 and the lower end of section 6 thereof. With the filter unit 3 located in position, the lowermost portion of section 7 of column 2 locates in the depression 13 in the upper surface of the filter 12 allowing the filtrate to come into proximity with the liquid inlet 8. As a result, the rate of filtration is enhanced.

CLAIMS

1. A method of obtaining a sample of a biomolecule from a suspension comprising a solution containing the biomolecule and insoluble material, the method comprising the steps of:

(a) providing a biomolecule purification assembly comprised of a vessel having a liquid inlet and of a filter unit removably located on the liquid inlet;

(b) effecting a filtration of the suspension through the filter unit so as to cause the solution to enter the vessel through the liquid inlet;

(c) removing the filter unit from the liquid inlet;

(d) immobilising the biomolecule on a solid phase support; and

(e) subjecting the biomolecule to at least one of the steps of washing on the support and elution from the support to obtain a purified sample of the biomolecule.

2. A method as claimed in claim 1 wherein the step (e) comprises washing the solid phase support and said support is contained within the vessel during this step.

3. A method as claimed in claim 2 wherein step (e) comprises eluting the biomolecule from solid phase support contained within the vessel, the elution being effected through said inlet of the vessel.

4. A method as claimed in any one of claims 1 to 3 wherein the step (b) the suspension to be filtered is contained in a well and in step (c) the filter unit is discharged into that well.

5. A method as claimed in any one of claim 1 to 4 wherein the biomolecule is absorbed onto the solid phase support in the presence of the chaotropic salt or other agent(s) for effecting said adsorption.

6. A method as claimed in claim 5 wherein, for the purposes of step (d) the filtrate in the vessel contains the chaotropic salt or other agent and the filtrate is discharged onto the support and the resultant mixture is taken back into the vessel.

7. A method as claimed in claim 5 wherein, for the purposes of step (d), the filtrate is discharged into a mixture of the support and the chaotropic salt or other agent(s) and the resultant mixture is taken back into the vessel.

8. A method as claimed in any one of claims 1 to 8 wherein the solid phase support comprises magnetic beads.

9. A method as claimed in any one of claims 1 to 8 wherein the vessel of the biomolecule purification assembly is an open-ended, vertically disposed column.

10. A method as claimed in claim 9 wherein the column has an upper bore section and a lower bore section of reduced diameter as compared to the upper section.

11. Apparatus for obtaining a sample of a biomolecule from a suspension comprising a solution of the biomolecule and insoluble material the apparatus comprising

(i) a filtration station at which is provided a biomolecule purification assembly comprised of a vessel having a liquid inlet and a removable filter unit located at the inlet of the vessel, said filtration station being provided with means for causing the

solution present in the suspension to pass through the filter unit and the liquid inlet into the vessel;

(ii) means for removing the filter unit from the vessel; and

(iii) at least one of a washing station and an elution station.

12. Apparatus as claimed in claim 11 comprising an elution station and a washing station.

13. Apparatus as claimed in claim 11 or 12 further comprising a solid phase support supply station preferably provided between (ii) and (iii).

14. An apparatus as claimed in any one of claims 11 to 13 wherein the vessel is an open-ended flow-through column.

15. An apparatus as claimed in claim 14 wherein the column has an upper bore section and a lower bore section of reduced diameter compared to the upper section.

16. An apparatus as claimed in claim 14 or 15 wherein the filter unit comprises a sleeve which incorporates the filter and which is located over the lower end of the column.

17. An apparatus as claimed in claim 16 wherein the upper surface of the filter has a depression and the lower end of the column locates in the depression.

18. An apparatus as claimed in any one of claims 11to 17 in which the pore size of the filter is in the range 0.2 to 50 microns.

19. An apparatus according to any one of claims 11to 18 in which the suspension to be filtered is contained in a well and once the filtration operation is complete the filter unit is automatically discarded into that well.

20. An apparatus according to claim 19 further comprising a stripping arrangement which acts on the filter unit when the assembly is moved upwards causing the filter unit to be discharged into the well.

21. An apparatus according to any one of claims 11to 20 which is capable of handling an array of biomolecule purification assemblies.

22. An apparatus according to claim 21 wherein each individual step of filtration, removal of the filtration unit washing and elution is adapted to be effected simultaneously on all members of the array.

23. A biomolecule purification assembly comprising a vessel having a liquid inlet and a filter unit removably located on the liquid inlet wherein the vessel is in the form of a column having a first bore section and a second bore section of reduced diameter as compared to the first section, and the filter unit is comprised of a sleeve which houses the filter and which is removably located on the end of the vessel remote from the first bore section.

24. A biomolecule purification assembly as claimed in claim 22 wherein the surface of the filter adjacent to the inlet of the vessel has a depression and the end of the vessel locates in the depression.

25. A method as claimed in claim 1 wherein the suspension is obtained by the steps of:

(i) providing a re-suspension of a bacterial pellet in solution;

(ii) adding lysis solution to the re-suspension to effect release of cellular contents; and

(iii) adding neutralising solution to the lysed re-suspension to effect the formation of a suspension comprising a solution of a biomolecule and insoluble material for use in the method of claim 1;

in which the lysis solution and/or neutralising solution are each added in multiple aliquots which are ejected downwardly into the re-suspension to provide the suspension in the absence of additional mechanical agitation or stirring without the dispensing apparatus coming into contact with the sample or the containing vessel.

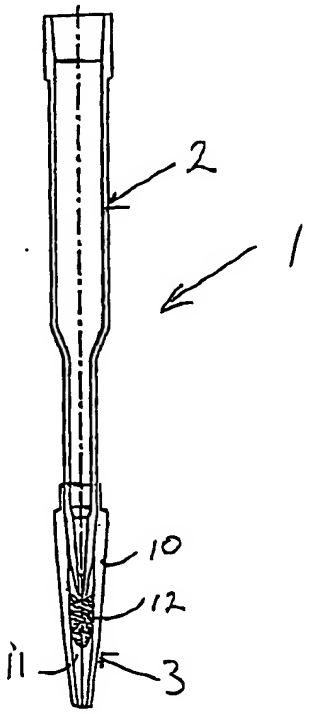

FIG 1

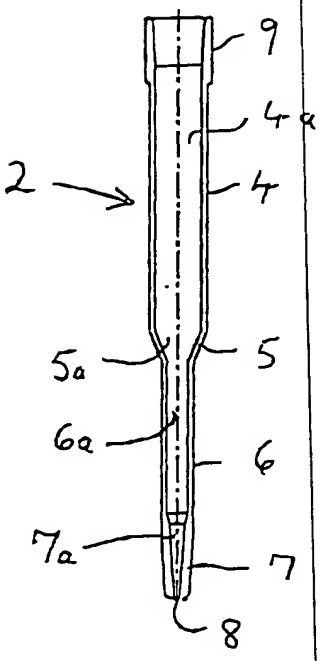

FIG 2

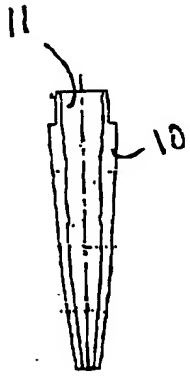

FIG 3

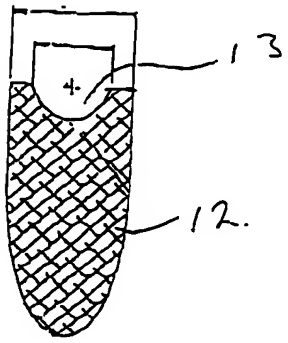

FIG 4

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